Diseases Caused by Bacteria and Phytoplasmas

First Report of ‘*Candidatus Liberibacter africanus*’ Associated with African Greening of *Citrus* in Angola

P. H. Fourie,^{1,2,†} W. Kirkman,¹ G. Cook,¹ C. Steyn,¹ R. de Bruyn,¹ R. Bester,³ R. Roberts,⁴ D. D. M. Bassimba,^{5,6} C. M. José,⁶ and H. J. Maree^{1,3}

¹ Citrus Research International, Nelspruit, South Africa

² Department of Plant Pathology, Stellenbosch University, Stellenbosch, South Africa

³ Department of Genetics, Stellenbosch University, Stellenbosch, South Africa

⁴ Agricultural Research Council – Plant Protection Research, Pretoria, South Africa

⁵ Instituto de Investigação Agronómica, Chianga, Huambo, Angola

⁶ PlantCare, Quarto C, Luanda, Angola

Plant Dis. 105:486, 2021; published online as <https://doi.org/10.1094/PDIS-06-20-1392-PDN>. Accepted for publication 21 August 2020.

Huanglongbing (HLB, Asian citrus greening), a devastating disease of citrus, has not been detected in southern Africa (Gottwald 2010). HLB is associated with ‘*Candidatus Liberibacter asiaticus*’ (CLAs), a phloem-limited bacterium vectored by *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), the Asian citrus psyllid (ACP). African citrus greening, associated with ‘*Candidatus Liberibacter africanus*’ (CLaf) and its vector the African citrus triozid, *Trioxa erythrae* (Del Guercio) (Hemiptera: Triozidae), are endemic to Africa, although not previously reported from Angola. African greening is less severe than HLB, largely due to heat sensitivity of CLaf and its vector. Introduction of HLB into southern Africa would be devastating to citrus production in commercial and informal sectors. There was concern CLAs or ACP might have inadvertently been introduced into Angola. In July 2019, a survey was conducted in two citrus nurseries in Luanda and Caxito and in different orchards on seven farms surrounding Calulo and Quibala. Yellow sticky traps for insects were placed at various sites and were collected after ~3 weeks. Breeding signs of *T. erythrae* (pit galls) were observed on citrus in some locations, but no insect vectors were detected on traps. Trees were inspected for signs and symptoms of citrus pests and diseases, particularly those resembling HLB (foliar blotchy mottle, shoot chlorosis, vein yellowing and corking, lopsided fruit with aborted seeds, and color inversion) and its vectors (pit galls on leaves or waxy exudates). Leaves and shoots with suspect symptoms were sampled for analysis (43 samples). DNA was extracted

from petiole and midrib tissue of leaves using a modified CTAB extraction protocol (Doyle and Doyle 1990). Real-time PCR was done using universal *Liberibacter* primers (Roberts et al. 2015), CLaf-specific primers (Li et al. 2006), and CLAs-specific primers (Bao et al. 2020). All real-time PCR protocols indicated the presence of CLaf in six samples. CLAs or other citrus *Liberibacter* species were not detected. Presence of CLaf in sample 37 was confirmed by constructing a library (Nextflex DNA Sequencing Kit, PerkinElmer) with extracted DNA and performing high-throughput sequencing on an Ion Torrent S5 platform (Central Analytical Facility, Stellenbosch University). To improve the quality of the reads, all 233,617,700 obtained reads were trimmed from the 3' end to a maximum length of 240 nt using Trimmomatic (Bolger et al. 2014). The high-quality reads were mapped to the *Citrus sinensis* reference genome (NC_023046.1) using Bowtie 2.3.4 (Langmead and Salzberg 2012) to subtract all the reads that had high identity to the host plant (number of mismatches allowed in the seed was set to 1). The 14,691,369 unmapped reads (6.2% of original data) were mapped to the CLaf reference genome NZ_CP004021.1 using CLC Genomics Workbench 10.1.1 (Qiagen) (length fraction = 0.8; similarity fraction = 0.9). A CLaf consensus genome was generated that spanned 99.7% of the reference genome, and the 163,001 mapped reads had a 22.9 mean read coverage. The consensus sequence was 99.7% identical to NZ_CP004021.1 and was submitted to GenBank as CP054879. The positive CLaf detections were from trees with typical HLB or African citrus greening symptoms in a commercial citrus farm outside Calulo and included two ‘Ponkan’ mandarin (*C. reticulata*), two Valencia, and one ‘Navelina’ tree (*C. sinensis*), and from a citrus nursery in Luanda (one lime tree; *C. aurantifolia*). This first report of CLaf in Angola highlights the need to prevent spread by removing infected trees and managing the insect vector, as well as the need for further surveys to determine the occurrence of African greening and its vectors in other provinces and to confirm the absence of exotic citrus pests and diseases.

References:

- Bao, M., et al. 2020. Plant Dis. 104:527.
- Bolger, A. M., et al. 2014. Bioinformatics 30:2114.
- Doyle, J. J., and Doyle, J. L. 1990. Focus 12:13.
- Gottwald, T. R. 2010. Annu. Rev. Phytopathol. 48:119.
- Langmead, B., and Salzberg, S. 2012. Nat. Methods 9:357.
- Li, W., et al. 2006. J. Microbiol. Methods 66:104.
- Roberts, R., et al. 2015. Int. J. Syst. Evol. Microbiol. 65:723.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: HLB, huanglongbing, greening, citrus

[†]Indicates the corresponding author.

E-mail: phf@cri.co.za (P.H.F.)